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HA1077, a protein kinase inhibitor, inhibits calponin phosphorylation on Ser^{175} in porcine coronary artery

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Abstract

Calponin is a thin filament-associated protein which has been implicated in the modulation of the contractile state of smooth muscle via its interaction with actin and inhibition of the actin-activated myosin Mg-ATPase. This inhibitory effect is alleviated by phosphorylation of calponin at Ser^{175} in vitro by protein kinase C. The issue of calponin phosphorylation in intact smooth muscle in response to agonists that activate protein kinase C is controversial. We have produced a monoclonal antibody that specifically recognizes calponin phosphorylated at Ser^{175} and used it to analyze calponin phosphorylation in porcine coronary arterial smooth muscle stimulated with prostaglandin $F_{2\alpha}$ or phorbol 12,13-dibutylate (PDB). Calponin phosphorylation increased rapidly in response to prostaglandin $F_{2\alpha}$ concomitant with the increase in tension. Calponin was then dephosphorylated while force was maintained. Tension development in response to PDB was significantly slower, but again calponin phosphorylation paralleled force development. In this case, calponin dephosphorylation was very slow, consistent with prolonged activation of protein kinase C. The protein kinase inhibitors, HA1077 (1-5-(isoquinoline sulfonyl)-homopiperazine HCl) and HA1100 (1-hydroxy HA1077; 1-(hydroxy-5-isoquinoline sulfonyl-homopiperazine), inhibited tension development and calponin phosphorylation in a concentration-dependent manner with similar ED_{50} values in response to prostaglandin $F_{2\alpha}$ and PDB. These results support physiological roles for calponin in force development in smooth muscle in response to agonists which trigger protein kinase C activation and in the latch state, i.e., force maintenance at low energy cost. Furthermore, the vasodilator effect of HA1077 and HA1100 is more likely due to inhibition of protein kinase C than of myosin light chain kinase.

Keywords: Calponin; Phosphorylation; Smooth muscle; Contraction; HA1077; HA1100

1. Introduction

Phosphorylation at Ser^{19} of the 20 kDa myosin light chain is necessary and sufficient to induce smooth muscle contraction (Walsh et al., 1982; Gagelmann et al., 1984; Itoh et al., 1989). Several reports, however, indicate a modulatory role for thin filament-associated proteins (Ebashi et al., 1980; Takahashi et al., 1988). Calponin (see Winder and Walsh, 1993, 1996; Gimona and Small, 1995 for reviews), specifically the α- or h1-isoform (Takahashi and Nadal-Ginard, 1991; Strasser et al., 1993), is a thin filament-associated protein (Walsh et al., 1993) that inhibits (i) the actin-activated Mg-ATPase activity of phosphorylated smooth muscle myosin (Winder and Walsh, 1990; Abe et al., 1990); (ii) the movement of fluorescent-labelled actin filaments over immobilized phosphorylated smooth muscle myosin (Shirinski et al., 1992; Haeberle, 1994) and (iii) force development or unloaded shortening velocity in permeabilized smooth muscle strips (Itoh et al., 1994; Jaworowski et al., 1995; Obara et al., 1996; Uyama et al., 1996). This inhibitory effect is reversed upon phosphorylation by protein kinase C (Winder and Walsh, 1990; Itoh et al., 1994; Pohl et al., 1997). Calponin phosphorylation has been demonstrated in intact smooth muscle in some studies in response to contractile agonists that activate protein kinase C (Winder et al., 1993; Carmichael et

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al., 1994; Gerthoffer and Pohl, 1994; Rokolya et al., 1994; Mino et al., 1995; Pohl et al., 1997), whereas others found no evidence of phosphorylation of calponin in various stimulated smooth muscles (Gimona et al., 1992; Bàràny and Bàràny, 1993; Adam et al., 1995).

Calponin is phosphorylated in vitro by protein kinase C at Ser^{175} (Winder et al., 1993) or Thr^{184} (Nakamura et al., 1993). Calponin phosphorylation by protein kinase C at Thr^{184} is inhibited by F-actin (Nakamura et al., 1993; Nagumo et al., 1994) but this does not appear to be the case for phosphorylation at Ser^{175} (Winder et al., 1993). The properties of site-specific mutants of calponin in which Ser^{175} was replaced by Ala, Asp or Thr support an important role for this residue in actin binding and actomyosin ATPase inhibition (Tang et al., 1996). To address the issue of calponin phosphorylation in intact muscle, we have raised an antibody to a synthetic peptide corresponding to residues 171–179 of calponin with phosphoserine at position 175 and used it to analyze calponin phosphorylation in intact porcine coronary arterial smooth muscle.

Isoquinolinesulfonamide derivatives are protein kinase inhibitors that bind competitively to the ATP binding sites of several protein kinases (Hidaka et al., 1984). HA1077 (1-5-(isoquinoline sulfonyl)-homopiperazine HCl) is a vasodilator that potently inhibits the activity of various protein kinases, including protein kinase C and myosin light chain kinase, in vitro (Asano et al., 1989). We have previously reported that HA1077 also inhibits agonist-induced contraction and myosin light chain phosphorylation in rabbit aorta (Seto et al., 1991). However, the concentration–response curves for inhibition of myosin light chain phosphorylation and tension by HA1077 were not identical: the ED_{50} value (the concentration of HA1077 required to produce 50% inhibition of myosin light chain phosphorylation or tension) was 2 μM for myosin light chain phosphorylation and 100 μM for tension when the muscle was stimulated with 40 mM K^+, and 2.1 μM for myosin light chain phosphorylation and 50 μM for tension when stimulated with 30 μM prostaglandin $F_{2\alpha}$ (Seto et al., 1991). The inhibitory effect of HA1077 on smooth muscle contraction cannot, therefore, be explained solely by inhibition of myosin light chain phosphorylation, suggesting that phosphorylation of another protein involved in the regulation of contraction must also be inhibited by HA1077.

HA1100 (1-hydroxy HA1077; 1-(hydroxy-5-isoquinoline sulfonyl-homopiperazinde) is also a potent vasodilator. This compound potently inhibits protein kinase C but is a much weaker inhibitor of myosin light chain kinase than is HA1077.

The aim of this study was to investigate the role of calponin phosphorylation at Ser^{175} as protein kinase C site in the generation of contractile force, by using the antibody specific for Ser^{175}-phosphorylated calponin and to clarify the mechanism of vasodilator effects of HA1077 and HA1100 through the inhibition of calponin phosphorylation.

2. Materials and methods

2.1. Tissue preparation

Fresh porcine hearts were obtained from a local abattoir and transported to the laboratory in ice-cold physiological saline solution (PSS) composed of (in millimoles): NaCl 115; KCl 4.7; $CaCl_2$ 2.5; $MgCl_2$ 1.2; $NaHCO_3$ 25; KH_2PO_4 1.2 and glucose 10.0, pH 7.4. The coronary artery was excised and segments were trimmed of fat and connective tissue.

2.2. Tension measurement

Tension was measured in strips of porcine coronary artery (5 mm × 2 mm) using an isometric force transducer (Nihon Kohden, TB-611T) as described by Seto et al. (1991).

2.3. Monoclonal antibody

A monoclonal antibody against phosphorylated calponin was raised with a synthetic peptide corresponding to residues 171–179 of calponin containing phosphorylated Ser^{175} as an antigen. The peptide sequence was Asn-Lys-Phe-Ala-Ser(PO_3H_2)-Gln-Gln-Gly-Met and a cysteine residue was added to the C-terminus for conjugation to keyhole lympet hemocyanin.The conjugated peptide was injected into BALB/c mice 4 times (once per week) and the spleen was removed. Clones producing a specific antibody against phosphorylated calponin were identified using calponin phosphorylated by protein kinase C. Another synthetic peptide, Gly-Thr(PO_3H_2)-Arg-Arg-His-Leu-Tyr-Asp-Pro-Lys-Leu-Gly-Thr, corresponding to residues 183–195 of calponin containing phosphorylated Thr^{184}, was used to confirm the specificity of the antibody.

2.4. Measurement of calponin phosphorylation

Arterial strips mounted for isometric force measurement were frozen by immersion in acetone containing 10% trichloroacetic acid and 10 mM dithiothreitol. The frozen tissues were washed twice with acetone containing 10 mM dithiothreitol to remove the trichloroacetic acid and dried. The dried strips were cut into small pieces, and protein was extracted with 100 μl of sodium dodecyl sulfate (SDS)-gel sample buffer containing 6 M urea, 0.5% SDS, 0.1% 2-mercaptoethanol, 0.01% bromophenol blue, 25 mM Tris–HCl (pH 7.5). The extract was passed through a 0.45 μm membrane filter and subjected to SDS-polyacrylamide gel electrophoresis (PAGE) as described by Laemmli (1970). Proteins were transferred to nitrocellulose membranes as described by Towbin et al. (1979). Phosphorylated calponin was visualized using a biotinylated secondary antibody, avidine-peroxidase, and ECL Western blotting detection reagents (Amersham). Phosphorylated

calponin was quantified by densitometry of the exposed X-ray film with a GS-700 Imaging Densitometer (Bio-Rad).

2.5. Measurement of inhibitory activity of HA1077 and HA1100

Inhibition constant (K_i) values of HA1077 and HA1100 on protein kinase C isozymes were determined by the method of Hidaka et al. Casein kinase II was partially purified as described previously (Sutherland et al., 1994). Caldesmon (0.1 mg/ml) was incubated at 30°C in 50 mM Tris–HCl (pH 7.5), 0.15 M KCl, 10 mM $MgCl_2$, 5–50 μM [γ-^{32}P] ATP with casein kinase II and various concentrations of HA1077 and HA1100. The reactions were stopped by the addition of 5% trichloroacetic acid and [^{32}P] incorporation was quantified by scintillation counting.

2.6. Materials

HA1077 was synthesized from 5-isoquinoline sulfonic acid (Morikawa et al., 1984). Prostaglandin $F_{2\alpha}$ was obtained from Nakalai Tesque (Tokyo, Japan). Phorbol 12,13-dibutyrate (PDB) was obtained from Sigma (St. Louis, MO, U.S.A.). Phosphorylated peptides were obtained from the Peptide Institute (Osaka, Japan).

3. Results

3.1. Specificity of anti-(Ser^{175}-phosphorylated calponin)

To obtain an antibody specific for Ser^{175}-phosphorylated calponin, a synthetic peptide corresponding to amino acid residues 171–179, containing phosphoserine at position 175, and coupled to keyhole lympet hemocyanine via a C-terminal Cys was used as an antigen. Fig. 1A shows an immunoblot of unphosphorylated calponin (lane 1) and calponin phosphorylated by protein kinase C (lane 2) using this antibody. The antibody recognized only phosphorylated calponin. For further confirmation of the specificity of the antibody, porcine coronary arterial smooth muscle (untreated or stimulated with 30 μM prostaglandin $F_{2\alpha}$ for 2 min) was homogenized and subjected to SDS-PAGE and transferred to a nitrocellulose membrane for immunoblotting with anti-(Ser^{175}-phosphorylated calponin). The antibody specifically recognized phosphorylated calponin of approximately 34 kDa based on its migration relative to prestained molecular weight standards (Fig. 1B). This band was confirmed to be calponin, using a polyclonal antibody specific for calponin (data not shown). Finally, cross-reactivity with a distinct synthetic peptide corresponding to amino acids 183–195 of calponin and containing phosphorylated Thr^{184} was examined by enzyme-linked immunosorbent assay (Fig. 1C). The antibody only recognized the 171–179 phosphopeptide and calponin phosphorylated by protein kinase C, confirming that the antibody is specific for Ser^{175}-phosphorylated calponin.

3.2. Calponin phosphorylation in intact smooth muscle strips

Calponin phosphorylation was analyzed in intact smooth muscle by Western blotting, using the anti-(Ser^{175}-phosphorylated calponin). Fig. 2A shows the time course of tension development in porcine coronary artery stimulated

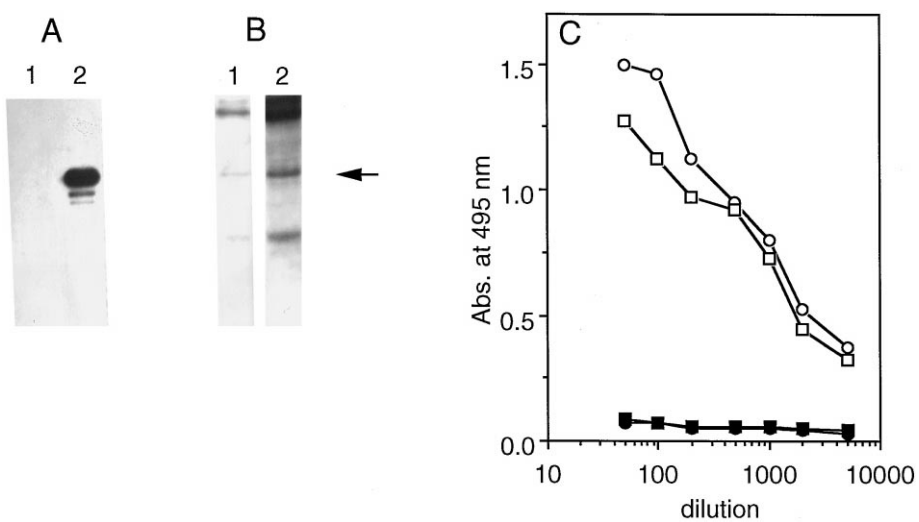

Fig. 1. Specificity of anti-(Ser^{175}-phosphorylated calponin). (A) Unphosphorylated calponin (lane 1) and phosphorylated calponin (lane 2) (1 μg each) were subjected to SDS-PAGE and immunoblotting with anti-(Ser^{175}-phosphorylated calponin). (B) Porcine coronary artery (control or stimulated for 2 min with 30 μM prostaglandin $F_{2\alpha}$) was homogenized and subjected to SDS-PAGE and immunoblotting with anti-(Ser^{175}-phosphorylated calponin). (C) The wells of a 96-well ELISA plate were coated with the synthetic peptide, calponin 171–179, containing phosphoserine^{175} (open circles), synthetic peptide, calponin 183–195, containing phosphothreonine^{184} (filled circles),purified calponin phosphorylated in vitro by protein kinase C (open squares), or purified unphosphorylated calponin (filled squares). Anti-(Ser^{175}-phosphorylated calponin) was added at the dilutions indicated and incubated for 30 min. The plate was washed with PBS containing 0.1% Tween 20 and incubated with secondary antibody conjugated to horseradish peroxidase.

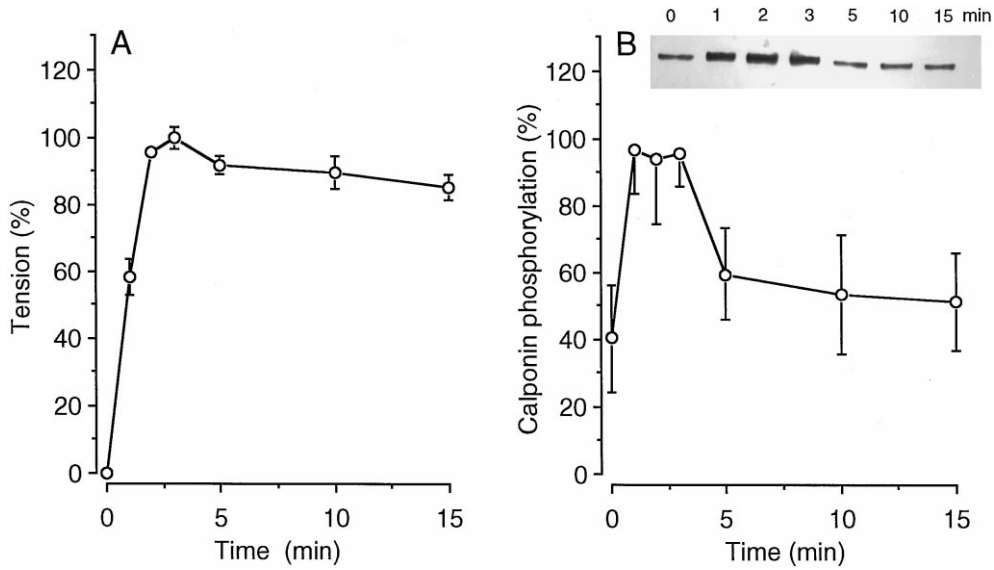

Fig. 2. Time courses of tension development (A) and calponin phosphorylation (B) in porcine coronary artery in response to 30 μM prostaglandin $F_{2\alpha}$. Inset I (panel B) shows a representative immunoblot. The quantitative data in panel (B) obtained by densitometric scanning of immnoblots, are given as means ± S.D. of four experiments.

with 30 μM prostaglandin $F_{2\alpha}$. Similar muscle strips were quick frozen at selected times following addition of prostaglandin $F_{2\alpha}$ and calponin phosphorylation was analyzed by immunoblotting with anti-(Ser^{175}-phosphorylated calponin) (Fig. 2B). The immunoblot from a representative experiment is shown in the inset of Fig. 2B and quantitative data from several experiments, obtained by densitometric scanning of immunoblots, are displayed graphically. Calponin phosphorylation increased rapidly following the addition of prostaglandin $F_{2\alpha}$, was maintained for ≈2 min, and then declined to resting levels.

Calponin phosphorylation was also examined in porcine coronary arterial smooth muscle strips stimulated with the membrane-permeable protein kinase C activator, PDB. Fig. 3A shows the time course of tension development in response to 100 nM PDB and the corresponding time course of calponin phosphorylation is shown in Fig. 3B. Both tension and calponin phosphorylation increased more

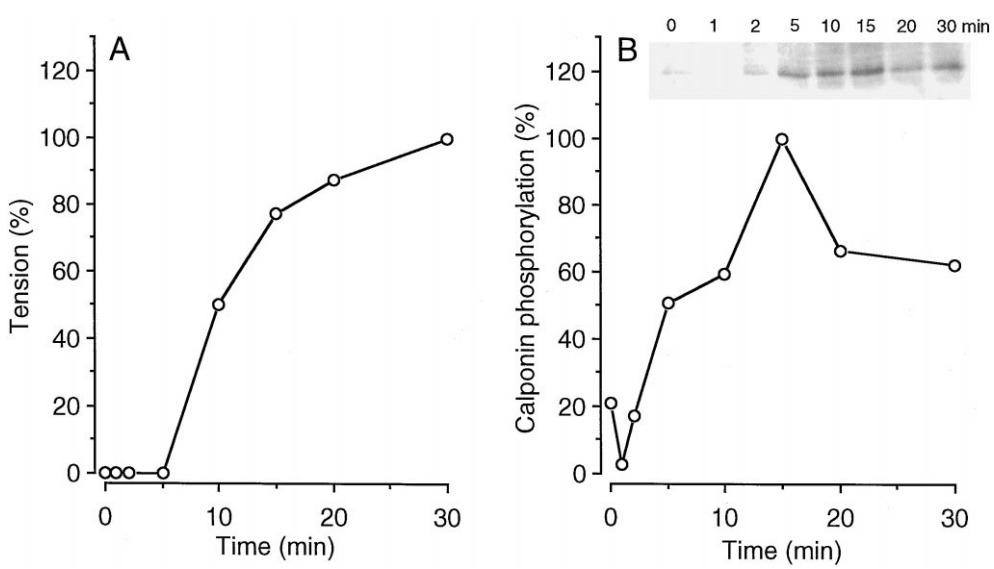

Fig. 3. Time courses of tension development (A) and calponin phosphorylation (B) in porcine coronary artery in response to 100 nM PDB. Panel (B) shows a representative immunoblot and the corresponding quantitative data obtained by densitometric analysis. Similar results were obtained in an independent experiment.

slowly in response to PDB than to prostaglandin $F_{2\alpha}$ but again there was a good correlation between tension development and calponin phosphorylation. In the case of PDB stimulation, however, calponin dephosphorylation was very slow, presumably reflecting prolonged activation of protein kinase C by phorbol ester. The transient effect of prostaglandin $F_{2\alpha}$ on calponin phosphorylation was probably due to metabolism of the prostaglandin. There is another possibility that the differential kinetics may result from the difference in an activation of different pools of protein kinase C isozymes.

3.3. Effects of HA1077 on calponin phosphorylation and tension generation

HA1077 and HA1100, isoquinolinesulfonamide derivatives, were potent inhibitors of protein kinase C with K_i values of 3.3 and 18 μM, respectively. HA1077 inhibited in an ATP-competitive manner the activity of protein kinase C isozymes preparations from rat brain, isozyme α, β and γ, with similar K_i values, 7.2, 7.0, 5.5 μM, respectively. HA1077 also inhibited myosin light chain kinase and casein kinase II with about 10-fold higher K_i (K_i: 36, and 21 μM, respectively) than did protein kinase C. This inhibitor shows highly potent inhibition against protein kinase A, and G with same K_i values of 1.6 μM (Seto et al., 1991), but scarcely inhibited the activity of tyrosine kinase preparation of human platelets, with K_i value of more than 100 μM (Y. Ozaki, Yamanasi Medical University, private communication). HA1100 has little effect on myosin light chain kinase, with a K_i value of more than 100 μM (Sakurada et al., 1998).

Prostaglandin $F_{2\alpha}$-induced tension and calponin phosphorylation in the presence of various concentrations of HA1077, added to the bath 15 min prior to prostaglandin $F_{2\alpha}$, are shown in Fig. 4A. Tension generation and calponin phosphorylation were measured 2 min after stimulation with 30 μM prostaglandin $F_{2\alpha}$ at which time these values were maximal (see Fig. 2). HA1077 attenuated both tension and calponin phosphorylation in a concentration-dependent manner and the ED_{50} values for inhibition of tension and calponin phosphorylation were 14 μM and 26 μM, respectively.

Similarly to prostaglandin $F_{2\alpha}$ stimulation, the tension and calponin phosphorylation induced by 100 nM PDB in the presence of various concentrations of HA1077 are shown in Fig. 4B. Tension generation and calponin phosphorylation were measured 15 min after stimulation with 100 nM PDB, at which time these values were maximal (see Fig. 3). Both tension and calponin phosphorylation were reduced by HA1077 in a concentration-dependent manner, with ED_{50} values of 19 μM and 36 μM, respectively, i.e., similar to the quantitative effects of HA1077 on prostaglandin $F_{2\alpha}$-induced tension and calponin phosphorylation.

The effect of HA1100 on prostaglandin $F_{2\alpha}$-induced (Fig. 5A) and phorbol ester-induced (Fig. 5B) contraction was also investigated. Both tension and calponin phosphorylation were reduced by HA1100 in a concentration-dependent manner. ED_{50} values for inhibition of 30 μM prostaglandin $F_{2\alpha}$-induced tension and calponin phosphorylation were 30 and 32 μM and those of 100 nM PDB-induced tension and calponin phosphorylation were 30 and 34 μM, respectively. The quantitative inhibitory effects of

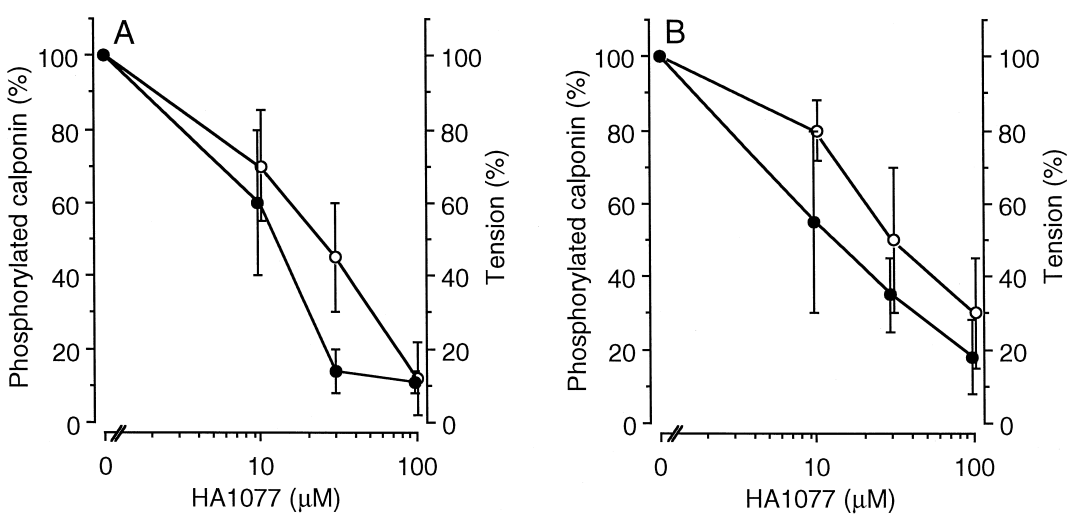

Fig. 4. Concentration-dependent inhibition by HA1077 of tension generation and calponin phosphorylation in porcine coronary artery stimulated with prostaglandin $F_{2\alpha}$ or PDB. HA1077 was applied at the concentrations indicated 15 min before the addition of 30 μM prostaglandin $F_{2\alpha}$ (A) or 100 nM PDB (B). Tension and calponin phosphorylation were measured 2 min (A) or 15 min (B) after stimulation. Tension (open circles) and calponin phosphorylation (filled circles) are presented as percentages of values obtained in the absence of HA1077. Each points is the mean ± S.D. of four experiments.

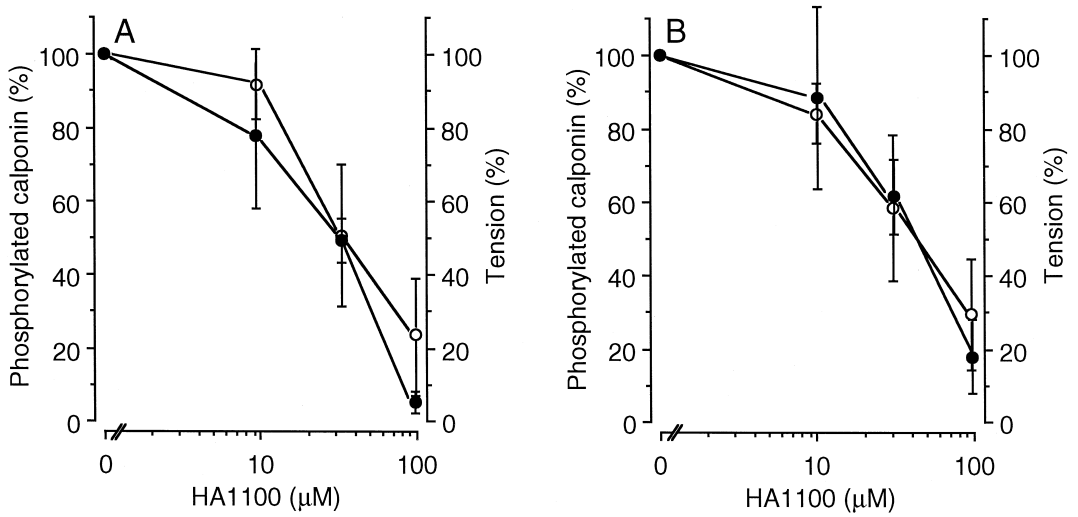

Fig. 5. Concentration-dependent inhibition by HA1100 of tension generation and calponin phosphorylation in porcine coronary artery stimulated with prostaglandin $F_{2\alpha}$ or PDB. HA1100 was applied at the concentrations indicated 15 min before the addition of 30 μM prostaglandin $F_{2\alpha}$ (A) or 100 nM PDB (B). Tension and calponin phosphorylation were measured 2 min (A) or 15 min (B) after stimulation. Tension (open circles) and calponin phosphorylation (filled circles) are represented as percentages of values obtained in the absence of HA1100. Each point is the mean ± S.D. of four experiments.

HA1100 on prostaglandin $F_{2\alpha}$-induced tension and calponin phosphorylation were similar to those on phorbol ester-induced tension and calponin phosphorylation.

4. Discussion

We have shown previously that there is a marked difference (25- to 50-fold) in the concentration-dependence of inhibition by the protein kinase inhibitor, HA1077, of force and of myosin light chain phosphorylation in rabbit aortic smooth muscle stimulated by K^+ depolarization or prostaglandin $F_{2\alpha}$ (Seto et al., 1991). This is consistent with numerous reports in the literature demonstrating the dissociation of myosin light chain phosphorylation and force generation (reviewed by Kargacin and Walsh, 1995), and indicates that mechanisms other than myosin light chain phosphorylation are involved in the regulation of smooth muscle contraction. One such mechanism involves the thin filament-associated protein, calponin, and its phosphorylation by protein kinase C at Ser^{175}. Calponin inhibits the actin-activated myosin Mg-ATPase, and this inhibition is prevented by phosphorylation by protein kinase C (Winder and Walsh, 1990) and restored upon dephosphorylation by type 2A protein phosphatase (Winder et al., 1992). We have investigated the potential involvement of calponin in the regulation of smooth muscle contraction by examining calponin phosphorylation in intact porcine coronary artery and the effect of HA1077 on both calponin phosphorylation and tension development in response to prostaglandin $F_{2\alpha}$ (which activates both myosin light chain kinase and protein kinase C) and PDB (a potent activator of protein kinase C).

The issue of calponin phosphorylation in vivo is controversial. Several instances have been reported of calponin phosphorylation in intact muscle strips in response to contractile agonists (carbachol, endothelin-1, phorbol esters) which activate protein kinase C (Winder et al., 1993; Carmichael et al., 1994; Gerthoffer and Pohl, 1994; Rokolya et al., 1994; Mino et al., 1995; Pohl et al., 1997). Several investigators, however, have failed to observe calponin phosphorylation in vivo (Gimona et al., 1992; Bàràny and Bàràny, 1993; Adam et al., 1995). The reason for these discrepancies is unclear, but may be related to the length of time of equilibration of tissue strips with [^{32}P]orthophosphate. We have found that an equilibration time of > 8 h is required to observe calponin phosphorylation in situ consistently. Another possibility remains: the phosphorylated calponin may leave the thin filaments and then may be exposed to 2A type phosphatase or Ca^{2+}-dependent protease. Thus, the detection of phosphorylated calponin may depend on the experimental conditions.

We therefore used a novel approach to address this issue. An antibody was produced which specifically recognizes calponin phosphorylated at Ser^{175} and which could be used in immunoblotting experiments to quantify changes in calponin phosphorylation in response to prostaglandin $F_{2\alpha}$ and PDB. This approach has the advantage of not requiring prior equilibration of the tissue with radiolabelled phosphate. In this way, low levels of phosphorylated calponin were detected in resting porcine coronary arterial smooth muscle, which is considered as a basal level. Stimulation with prostaglandin $F_{2\alpha}$ caused a significant increase in calponin phosphorylation within 1 min, which was maintained for ≈ 2 min before declining to

near resting levels at 5 min. The increase in calponin phosphorylation upon stimulation correlated with force development, whereas the subsequent decline in calponin phosphorylation correlated with the phase of force maintenance. This is exactly what could have been predicted on the basis of the known effects of calponin on the actin-activated myosin ATPase, i.e., inhibition by unphosphorylated calponin and no effect of phosphorylated calponin. Upon stimulation with prostaglandin $F_{2\alpha}$, myosin and calponin are rapidly phosphorylated, resulting in maximally actin-activated myosin Mg-ATPase and force development. Subsequent force maintenance occurs with low energy expenditure due in part to calponin dephosphorylation and in part to myosin dephosphorylation, latch bridges (Hai and Murphy, 1988) accounting for the maintained force.

In the case of stimulation with PDB, both calponin phosphorylation and tension development increased slowly, presumably due to specific activation of protein kinase C, in this case without an increase in myosin light chain phosphorylation. Slow tension development could be accounted for by prevention of the calponin inhibition of the actin-activated myosin Mg-ATPase (cross-bridge cycling rate), which unmasks a basal actomyosin ATPase activity due to the low level of myosin light chain phosphorylation ($\approx$ 0.1 mol Pi/mol myosin light chain) in a resting smooth muscle (Jiang and Morgan, 1989; Seto et al., 1991).

Although two agonists showed quite different modes of calponin phosphorylation and tension development, presumably depending on the properties of the agonists, there was good correlation between the time courses of two determinants in each experiment. These results suggest that calponin phosphorylation contributes to tension development and latch bridge formation.

HA1077 is an isoquinolinesulfonamide non-specific protein kinase inhibitor which acts competitively with respect to ATP. This compound inhibited force development in porcine coronary arterial smooth muscle in response to prostaglandin $F_{2\alpha}$ or phorbol ester in a concentration-dependent manner. HA1077 exhibited a similar concentration-dependent inhibition of calponin phosphorylation due to the same contractile agonists, supporting a role for calponin phosphorylation in force generation. This contrasts with the marked difference in inhibitory potency of HA1077 toward myosin light chain phosphorylation and tension (Seto et al., 1991) and suggests that inhibition of protein kinase C rather than of myosin light chain kinase accounts for the vasodilator effects of HA1077. A related compound, HA1100, inhibited force and calponin phosphorylation with similar potency (ED_{50}; 30 μM). This compound is known to inhibit protein kinase C but has little effect on myosin light chain kinase, supporting the conclusion drawn from the HA1077 experiments that inhibition of protein kinase C rather than of myosin light chain kinase, with reduced calponin phosphorylation, is the basis of the relaxant effects of HA1100.

Particularly, with PDB stimulation, calponin phosphorylation is reduced by HA1100, and myosin light chain phosphorylation hardly increases from its basal level, suggesting that one of the sites of HA1100 as well as of HA1077 action may be on a thin filament-involved pathway which is associated with protein kinase C activation followed by calponin phosphorylation. However, we could not exclude the possibility that HA compounds inhibit other protein kinases which contribute indirectly to tension development and calponin phosphorylation or dephosphorylation because these compounds have a wide spectrum of action against several kinds of protein kinases. Furthermore, we demonstrated, using the phosphorylation site-specific antibody against Ser^{175}-phosphocalponin that calponin phosphorylation at Ser^{175} occurs in arterial smooth muscle stimulated with prostaglandin $F_{2\alpha}$ and PDB.

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